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Dihydroorotate dehydrogenase arises from novel fused gene product with aspartate carbamoyltransferase in *Bodo saliens*

Takeshi Annoura [a], Idalia Sariego [a], Takeshi Nara [a], Takashi Makiuchi [a], Tsutomu Fujimura [b], Hikari Taka [b], Reiko Mineki [b], Kimie Murayama [b], Takashi Aoki [a,*]

[a] *Department of Molecular and Cellular Parasitology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*
[b] *Division of Proteomics and Biomolecular Science, Biomedical Research Center, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*

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Abstract

The *ACT–DHOD* gene in the kinetoplastid *Bodo saliens* encodes aspartate carbamoyltransferase and dihydroorotate dehydrogenase, the second and fourth enzymes of pyrimidine biosynthesis. Although the single mRNA species yielded a 70-kDa ACT–DHOD protein, Western blotting with anti-DHOD-peptide antibody showed a major band of 35-kDa and minor bands. In-gel digestion and liquid chromatography–tandem mass (MS/MS) spectrometry showed that the 35-kDa band contained DHOD-specific polypeptides and an ACT-specific polypeptide, suggesting the occurrence of independent DHOD and ACT. Immunoprecipitation and MS/MS analysis identified a 70-kDa ACT–DHOD and a 35-kDa DHOD independently, and the N-terminal amino acid of 35-kDa DHOD was blocked. In vitro processing assay showed that recombinant ACT–DHOD was decreased by the *B. saliens* lysate, accompanying the appearance of 35-kDa DHOD and 35-kDa ACT. These results indicate that fused ACT–DHOD is the precursor to mature DHOD. Large amount of 35-kDa DHOD in *B. saliens* is discussed from a viewpoint of its physiological roles.
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Keywords: Post-translational processing; Aspartate carbamoyltransferase; Dihydroorotate dehydrogenase; Fumarate reductase; Kinetoplastid; *Trypanosoma*; Bodonid; *Bodo saliens*; In vitro translation; Liquid chromatography–tandem mass spectrometry

The trypanosomatids and bodonids belong to the kinetoplastids, some of which cause human diseases such as Chagas' disease (*Trypanosoma cruzi*), African sleeping sickness (*T. brucei* group), and leishmaniasis (*Leishmania* spp.). Most bodonids are free-living organisms, while there is no free-living trypanosomatid reported so far. Phylogenetic studies revealed that trypanosomatids and bodonids may have evolved from a common euglenozoan ancestor [1,2]. Such a closer relationship suggested that a free-living bodonid could be used as a good model for studying an initial stage of establishing parasitism.

The de novo pyrimidine biosynthetic pathway consists of six enzymes for the production of uridine 5′-monophosphate (UMP). Previously, we reported a unique pyrimidine biosynthetic gene cluster that contains all the six genes [3], as a polycistronic transcription unit, in *Trypanosoma* and *Leishmania*. Dihydroorotate dehydrogenase (DHOD) is the fourth enzyme in the pathway catalyzing the oxidation of dihydroorotate to orotate. In these trypanosomatids, DHOD is classified as a family 1A enzyme, localized in the cytosol, and serves as a major soluble fumarate reductase [4]. The *DHOD* gene lies upstream to aspartate carbamoyltransferase (*ACT*) gene with a non-cording region

Abbreviations: ACT, aspartate carbamoyltransferase; DHOD, dihydroorotate dehydrogenase; UMP, uridine 5′-monophosphate; CAD, carbamoyl phosphate synthetase II–aspartate carbamoyltransferase–dihydroorotase; FMN, flavin mononucleotide; FRD, fumarate reductase; DIG, digoxigenin; ESI-Q-TOF, electrospray ionization-quadrupole-time-of-flight; LC–MS/MS, liquid chromatography–tandem mass spectrometry; CNBr, cyanogen bromide.

* Corresponding author. Fax: +81 3 5800 0476.
E-mail address: tksaoki@med.juntendo.ac.jp (T. Aoki).

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between them within the pyrimidine biosynthetic gene cluster in *T. cruzi* [5–7]. The DHOD is essential for fumarate reductase activity in *T. cruzi* and may be important in its adaptation to anaerobic conditions [8]. Thus, *T. cruzi* DHOD would function in pyrimidine biosynthesis and in fumarate redox homeostasis.

In general, eukaryotic enzymes catalyzing continuous or coupled reactions occur as multifunctional proteins, including dihydrofolate reductase (DHFR) and thymidylate synthase (TS) (DHFR–TS) [9,10]; carbamoyl phosphate synthetase II (CPS II), ACT, and dihydroorotase (DHO) (CAD) [11,12]; and orotate phosphoribosyltransferase (OPRT) and orotidine-5′-monophosphate decarboxylase (OMPDC) (OPRT–OMPDC) [13,14]. These proteins should have the advantage of channeling substrates and products in continuous enzymatic reactions. Exceptionally, however, we found a novel fused gene *ACT–DHOD* in *Bodo saliens* that may encode a multifunctional protein, ACT and DHOD (ACT–DHOD) [8], the second and fourth enzymes of de novo pyrimidine biosynthesis, lacking the third enzyme.

In the present study, we show that the *B. saliens ACT–DHOD* gene is transcribed to *ACT–DHOD* mRNA, translated to the single protein, ACT–DHOD, and finally converted to mature independent DHOD. The physiological roles of the mature enzyme that resembles the trypanosomatid DHOD are also discussed.

Materials and methods

Materials. Bodo saliens (ATCC 50358) was cultured at 25 °C in 500-cm^2 flasks (No. 132867, Nalge Nunc International, Denmark), each containing 250 ml of artificial seawater. They were fed with *Klebsiella pneumoniae* subsp. (ATCC 27889) every other day, and charged with mixed gas (5% O_2 and 5% CO_2 in N_2) in an anaerobic chamber every day. They were harvested and washed as described [8].

Northern blotting. Total RNA was prepared from *B. saliens* using TRIZOL reagent (Invitrogen, San Diego, CA) according to the manufacturer's protocol. Aliquots (10 μg RNA) were electrophoresed on 1% agarose/2% formaldehyde gels, blotted to nylon membranes (Roche, Mannheim, Germany), prehybridized for 2 h at 42 °C in DIG Easy Hyb solution (Roche), and hybridized for 16 h in the same solution containing DIG-labeled DNA probes (10 ng/ml) corresponding to full-length *ACT–DHOD* (nucleotides 1–1944), *ACT* (nucleotides 1–933), and *DHOD* (nucleotides 1000–1944) [3,8,15]. Membranes were washed and signal was detected using CSPD chemiluminescence detection system (Roche) [15].

Antigens and antibodies. We synthesized polypeptides, N-CPLPRNEE LSTDVDGDRR-C and N-KSCTAQQRDGNPAPR-C, specific for the *B. saliens* ACT and DHOD domains, respectively, which had been cysteine cross-linked to keyhole limpet hemocyanin. *B. saliens* recombinant DHOD (V334-E648) gene was amplified by PCR using the primers, 5′-CA CCGTGGACCTGAGCGTGAGC-3′ (sense) and 5′-TTACTCGATGAC CTTGAGCTT-3′ (antisense), and KOD-Plus-DNA polymerase. The PCR product was inserted in Champion pET100 Directional TOPO Expression system (Invitrogen). The recombinant DHOD was produced and affinity-purified as described [5]. Female Japanese white rabbits (for anti-peptide antibody) or female BALB/c mice (for anti-whole-DHOD antibody) were subcutaneously injected with individual antigens (200 μg peptide per rabbit or 100 μg DHOD per mouse) emulsified with Freund's complete adjuvant and boosted several times at 2-week intervals with 100 μg of corresponding antigens emulsified with Freund's incomplete adjuvant.

Immunoprecipitation and Western blotting. Immunoprecipitation was performed against the *B. saliens* cytosolic fraction (200 μg protein), prepared as described [8], using Protein G Magnetic Beads (New England Biolabs, Beverly, MA). Aliquots of the cytosolic fraction were mixed with equal volumes of SDS sample loading buffer, incubated at 95 °C for 3 min and applied to 4–20% gradient SDS–polyacrylamide gels. After the electrophoresis, the proteins were transferred to nitrocellulose membranes, blocked in 2% fetal calf serum (FCS) plus 0.05% Tween-PBS (PBST), incubated overnight at 4 °C with purified polyclonal antibody (1:1000 in blocking solution), and washed with PBST. The membranes were incubated with alkaline phosphatase-conjugated anti-rabbit or anti-mouse IgG antibody (1:3000 each, Bio-Rad Laboratories, CA) and washed with PBST, and signals were visualized with CSPD chemiluminescence detection system (Roche).

In-gel digestion and electrospray ionization-quadrupole-time-of-flight tandem mass spectrometry (ESI-Q-TOF MS/MS). In-gel digestion by trypsin of SDS–PAGE bands was performed as described [16]. Tryptic peptides were extracted, the solution was evaporated, and the peptides were dissolved in 10 μl 1% formic acid. Mass spectrometry was performed in AB-QSTAR pulsar *i* hybrid (Applied Biosystems, Framingham, CA) combined with a microliquid chromatograph (Magic 2002; Michrom Bioresources, Abum, CA) equipped with a 0.2-mm ID × 50 mm Magic C18 column. Amino acid sequences of tryptic peptides were examined by ESI-Q-TOF MS/MS using Mascot search engine (www.matrixscience.com/search_form_select.html) for all peptide mass mapping and MS/MS ion searching, against the proteome data base, NCBInr.

N-terminal sequence analysis. Electrophoresed proteins were transferred to nitrocellulose membranes and subjected to automated Edman degradation using a Hewlett Packard model G1005A protein sequencer

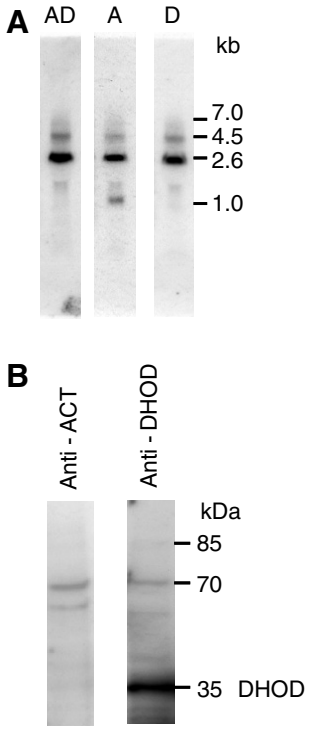

Fig. 1. Northern and Western blot analyses of *Bodo saliens* transcripts and proteins. (A) Northern blot analysis of *B. saliens* transcsripts. Aliquots of 10 μg total RNA were electrophoresed on 1% agarose gels, transferred to nylon membranes, and hybridized with DIG-labeled probes specific for *ACT* (lane A), *DHOD* (lane D), and full-length *ACT–DHOD* (lane AD). (B) Detection of 35-kDa DHOD and 35-kDa ACT proteins in *B. saliens* extract. Cytosolic fraction (50 μg protein) was resolved on SDS–PAGE and Western blotted with anti-ACT-peptide or anti-DHOD-peptide antibody. kDa, kilodaltons.

(Palo Alto, CA) attached to a HP1090 M liquid chromatograph as a PTH (phenylthiohydantoin) amino acid analyzer [16]. The same membrane was used for cyanogen bromide (CNBr)-digestion sequence analysis. The membrane was washed twice with 10% acetonitrile plus 0.1% TFA, and digestion was carried out in 100% formic acid containing 0.03 g/200 μl of CNBr at room temperature for 90 min. Samples were washed 3 times with distilled water, and amino acid sequences were determined as described.

In vitro expression and processing assay. The recombinant FLAG–ACT–DHOD–MYC was prepared in vitro using TNT SP6 High-Yield Protein Expression System (Promega). The DNA fragment carrying the *FLAG–ACT–DHOD–MYC* gene was amplified by PCR using the primers, 5′-GGGTGCGATCGCCATGGACTACAAAGACGATGACGACAA GATGCAGGGCACCCTCCAGGCGC-3′ (sense) and 5′-TTCTGTTT AAACCAGATCCTCTTCTGAGATGAGTTTTGTTCTTGCTCGA TGACCTTGAGCTTGCC-3′ (antisense), and KOD-Plus-DNA polymerase. The PCR product was digested with *Sgf*I and *Pme*I, and cloned into the protein-coding region (barnase site) of pF3A WG (BYDV) Flexi vector. The resulting recombinant plasmid, designated pF3A/FLAG–ACT–DHOD–MYC, was used for the in vitro cell-free expression system. Protein synthesis was initiated by adding the appropriate DNA template and allowed to proceed for 4 h at 25 °C in the presence or absence of flavin mononucleotide (FMN: 100 μM). The synthesized recombinant protein was incubated with *B. saliens* lysate, with or without a high concentration of protease inhibitor cocktail (Complete Mini Roche, with 1% Triton-X 100) at 25 °C, and aliquots (25 μg protein) were withdrawn at indicated times, followed by SDS–PAGE and Western blotting. FLAG-tagged proteins were detected by incubation with 1:2000 dilution of the primary FLAG M2 mouse monoclonal antibody (Sigma). MYC-tagged proteins were detected following the manufacturer's protocol (Sigma).

Results

Northern blot analysis of *B. saliens* total RNA, using DIG-labeled DNA probes specific for full-length *ACT–DHOD*, *ACT*, and *DHOD* showed that all three probes bound strongly to a 2.6-kb band, probably representing full-length *ACT–DHOD* mRNA (Fig. 1A). The open reading frame of the *ACT–DHOD* gene consists of 1944 bp [8], and thus, the other region, the sum of the spliced leader sequence and 3′ UTR may become approximately 650 bp. Prolonged development did not show any smaller bands, indicating the absence of independent *ACT* and *DHOD* transcripts. A quite faint band of 1.1-kb in lane A did not seem to be an independent *ACT* transcript, since the putative *ACT* gene (933 bp; [8]) plus above 650 bp should exhibit a 1.6-kb band. These results strongly suggested that there is neither independent *ACT* nor *DHOD* gene.

Western blotting of the *B. saliens* cytosolic fraction with anti-ACT-peptide antibody yielded a strong band at 70-kDa and faint bands at 60- and 35-kDa, whereas blotting with anti-DHOD-peptide antibody resulted in a strong band at 35-kDa and faint bands at 70- and 85-kDa (Fig. 1B). Extensive searches for ACT- or DHOD-specific tryptic peptides in the 60-, 70-, and 85-kDa bands by LC-MS/MS did not detect these polypeptides, which may have been due to the crude extract used in this experiment. Surprisingly, MS/MS analysis of tryptic peptides from the 35-kDa band detected a polypeptide, D592-K612, specific for the *B. saliens* DHOD domain (Table 1), as well as an ACT-specific polypeptide in the same 35-kDa band (Fig. 1B and Table 1). Although signal intensities of the spectra were weak, delta values (experimental minus calculated M_r) were 0.04 and 0.07 for the ACT- and DHOD-inner peptides, respectively (Table 1). These results suggest that *B. saliens* possesses independent ACT and DHOD proteins, each of molecular mass 35 kDa.

Immunoprecipitation of *B. saliens* DHOD protein with anti-DHOD-peptide antibody resulted in a 70-kDa band, visualized by both anti-ACT-peptide and anti-DHOD-peptide antibodies (Fig. 2A), likely representing full-length ACT–DHOD protein. MS/MS analysis of the 70-kDa protein showed seven polypeptides, six specific for ACT and one for DHOD (Table 1). This discrepancy in peptide number may have been due to the presence of many hydrophobic amino acids in ACT [8], resulting in their higher affinity binding to the liquid chromatography column. Typical MS/MS spectra of the DHOD- and ACT-specific polypeptides had higher intensity and lesser delta values of M_r

Table 1
Amino acid sequences of polypeptides, identified by liquid chromatography–tandem mass spectrometry, specific for *Bodo saliens* DHOD and ACT in the cytosolic fraction and in the immunoprecipitated 35-, 70-, and 85-kDa proteins

Fraction	Molecular mass	Enzyme	Sequence	M_r (expt)	M_r (calc)
Cytosolic	35-kDa	ACT	^{22}TILEDLTALALHLK^{35}	1549.87	1549.91
		DHOD	^{592}DAYQHLLAGASLVQVGTQLWK^{612}	2297.15	2297.22
Immunoprecipitated with anti-DHOD-peptide antibody	70-kDa	ACT	^{9}GAHIAGASQYNR^{21}	1243.63	1243.61
			^{22}TILEDLTALALHLK^{35}	1549.93	1549.91
			^{74}LGGSVVALPIEASSVSK^{90}	1612.89	1612.9
			^{99}TMDAYSDVIVLR^{111}	1381.74	1381.69
			^{161}TVVLVGDLK^{169}	942.59	942.57
			^{173}TVHSLAR^{179}	782.46	782.44
		DHOD	^{579}VIIGCGGVLCGR^{591}	1287.71	1287.68
Immunoprecipitated with anti-whole-DHOD antibody	35-kDa	DHOD	^{485}YLEAVTAVYPRPFGVK^{500}	1808.98	1808.98
			^{592}DAYQHLLAGASLVQVGTQLWK^{612}	2297.2	2297.22
			^{621}IRDELQAHLAR^{631}	1320.72	1320.73
	85-kDa	DHOD	^{579}VIIGCGGVLCGR^{591}	1287.74	1287.68

M_r (expt) and M_r (calc) denote the experimentally determined and calculated molecular masses, respectively. Delta values (experimental minus calculated) of less than 0.1 are considered highly specific.

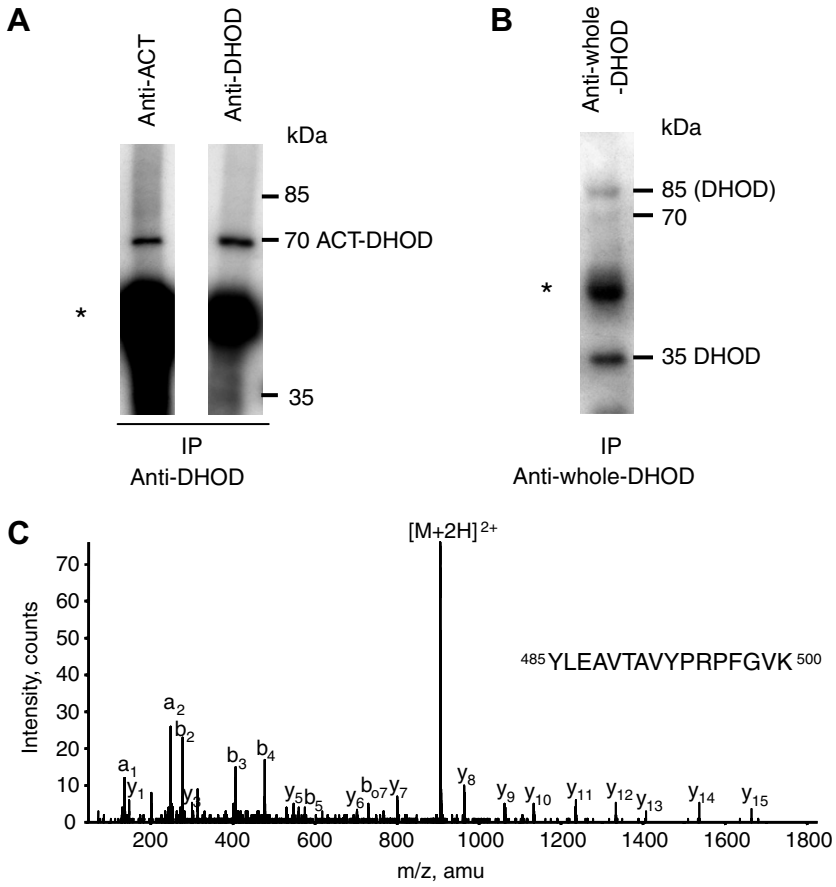

Fig. 2. Identification of the 70-kDa ACT–DHOD and the 35-kDa DHOD protein from *Bodo saliens*. (A) Immunoprecipitation of the 70-kDa ACT–DHOD protein from *B. saliens*. Cytosolic fraction (200 μg protein) was incubated with anti-DHOD-peptide antibody (5 μg) and the immunoprecipitated proteins were Western blotted using anti-ACT-peptide or anti-DHOD-peptide antibody. The asterisk, with a strong signal, was confirmed as the precipitated antibody used. (B) Immunoprecipitation of the 35-kDa DHOD protein was carried out using the anti-whole-DHOD antibody. The precipitated proteins were Western blotted using anti-whole-DHOD antibody. (C) Identification of a DHOD-specific amino acid sequence from the immunoprecipitated 35-kDa band (B), in MS/MS spectra of tryptic peptides Y485-K500.

(Table 1 and Supplemental Fig. 4S, A and B). These results indicate that the 2.6-kb *ACT–DHOD* mRNA (Fig. 1A) may be translated to a single, full-length ACT–DHOD protein of molecular mass 70-kDa. This 70-kDa primary translation product may be converted into 35-kDa DHOD in *B. saliens*.

We prepared a polyclonal antibody against the purified recombinant DHOD (V334–E648) to immunoprecipitate the 35-kDa DHOD. This antibody revealed two specific bands, at 35- and 85-kDa, and a faint band at 70-kDa (Fig. 2B). MS/MS analysis of the 35-kDa band demonstrated three tryptic polypeptides specific for DHOD with strong intensities and precise MS/MS spectra (Fig. 2C and Table 1), with one of these polypeptides, D592–K612, also seen in the cytosolic 35-kDa band (Table 1). In tryptic peptides from 85-kDa band (Fig. 2B), we detected only one MS/MS spectrum (^{579}VIIGCGGV LCGR^{591}) (Table 1 and Fig. 4S, C), the same polypeptide detected in the DHOD domain in immunoprecipitated 70-kDa ACT–DHOD (Table 1 and Fig. 4S, A). Thus, we expect that the 85-kDa protein may consist of 70-kDa ACT–DHOD protein and an additional protein (or polypeptide), of molecular mass 10- to 15-kDa. However, we could not exclude the possibility that the 85-kDa protein contains DHOD and a moiety of higher molecular mass ~50 kDa.

To determine the N-terminal sequence of 35-kDa DHOD, we analyzed proteins immunoprecipitated with anti-whole-DHOD antibody (Fig. 2B) using automated Edman degradation, but we detected no amino acid, indicating that the N-terminal amino acid of 35-kDa DHOD is blocked and that the 35-kDa protein is not an artificial degradation product of ACT–DHOD. Using the CNBr-digestion method, sequence analysis of the same membrane harboring 35-kDa band detected two independent DHOD-inner sequences (^{404}GLPNEGYEYY^{413} and ^{508}AHFD-QAAEVL^{517}). It is thus highly likely that the 35-kDa DHOD is a mature enzyme in vivo in *B. saliens*.

To confirm experimentally that ACT–DHOD is the precursor to mature DHOD, we prepared the recombinant ACT–DHOD, using an in vitro expression system in the presence and absence of FMN, the cofactor for DHOD. The FLAG- and MYC-tagged recombinant protein, FLAG–ACT–DHOD–MYC, was incubated with *B. saliens*

lysate, with or without added protease inhibitors. The FLAG–ACT–DHOD–MYC was decreased time-dependently, in the absence of inhibitors, and 35-kDa FLAG–ACT and 35-kDa DHOD–MYC appeared (Fig. 3A and B). Addition of FMN did not affect the increase in FLAG–ACT and DHOD–MYC. The processing from 70-kDa ACT–DHOD to independent ACT and DHOD was completely blocked by the high concentration of protease inhibitor cocktail with Triton-X (Fig. 3A and B). These results indicated that the 35-kDa DHOD arose from the fused ACT–DHOD in *B. saliens*.

Bodo saliens DHOD domain of ACT–DHOD was determined by alignment with trypanosomatid DHODs. The size of the purified recombinant DHOD (V334–E648) is about 35-kDa, and the protein showed yellow color, suggesting the presence of co-factor FMN. Its activity was determined by measuring orotate production in the presence of electron acceptors (with fumarate at 300 nm or with ubiquinone-1 at 287 nm), as described [4,5]. The recombinant protein revealed the high DHOD activity despite of a partial protein of ACT–DHOD (Table 2), depending on fumarate as electron acceptor that resembles the recombinant *T. cruzi* DHOD activity.

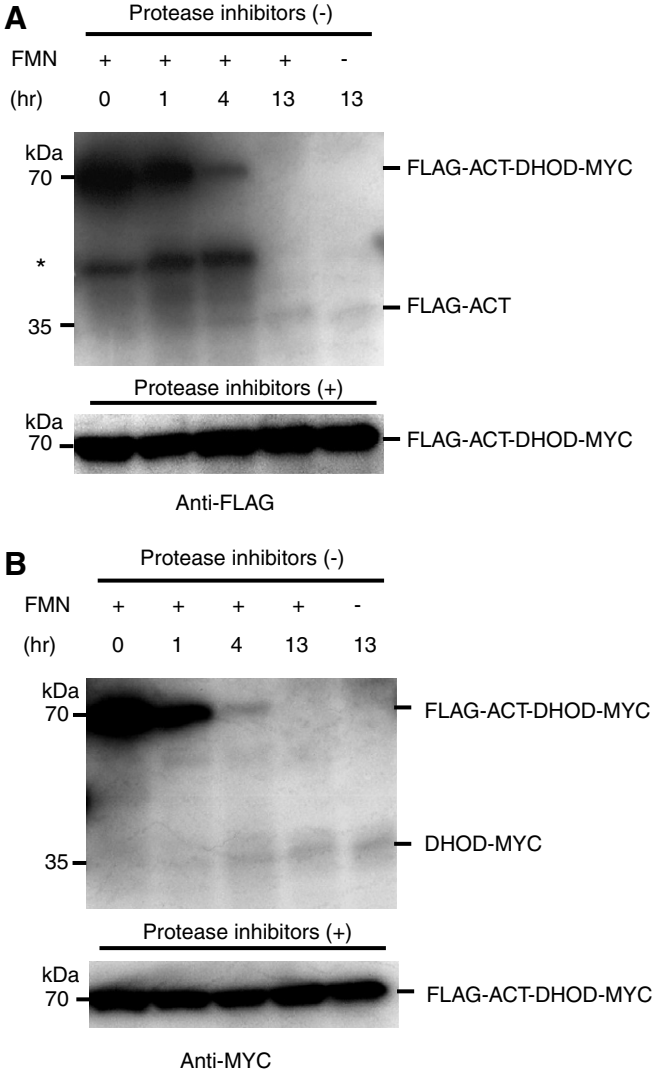

Fig. 3. In vitro processing of the recombinant FLAG–ACT–DHOD–MYC. The recombinant protein was incubated with *Bodo saliens* lysate in the absence or presence of the protease inhibitors. FMN, flavin mononucleotide. Aliquots (25 μg proteins) were withdrawn at 1, 4, and 13 h, and subjected to Western blot analysis using anti-FLAG (A) or anti-MYC (B) antibody. The asterisk is a non-specific band common to kinetoplastids.

Table 2
Comparison of activities of *Bodo saliens* and *Trypanosoma cruzi* recombinant DHODs

Enzyme source	Activities ($nmol^{-1}$ min^{-1} mg^{-1}) with fumarate (500 μM)	Activities ($nmol^{-1}$ min^{-1} mg^{-1}) with ubiquinone-1 (20 μM)
B. saliens (V334-E648)	3318 ± 1.7	987 ± 3.6
T. cruzi	2950 ± 2.1	840 ± 3.3

Assays were performed at 25 °C. Enzyme activities are shown as means ± SD of three independent measurements. *T. cruzi* DHOD activities are cited from [4].

Discussion

We have shown here that, in the kinetoplastid protist *B. saliens*, the *ACT–DHOD* gene is transcribed to a single *ACT–DHOD* mRNA, that its primary translation product is ACT–DHOD, that 35-kDa DHOD arose from ACT–DHOD in an in vitro processing assay, and finally that post-translational processing results in the production of N-terminal blocked mature DHOD. To our knowledge, the *ACT–DHOD* fused gene product and its maturation process is reported here for the first time, but the final product of *B. saliens* 35-kDa DHOD is highly homologous to the *T. cruzi* DHOD [8], the latter showing no such a maturation process.

Western blotting of *B. saliens* extract with anti-ACT-peptide and anti-DHOD-peptide antibodies resulted in a strong 70-kDa band and weak 60- and 35-kDa bands with the former, and a strong 35-kDa band and weak 70- and 85-kDa bands with the latter. These faint bands may carry DHOD or ACT protein. Immunoprecipitation and MS/MS analysis demonstrated the presence of DHOD in the 70- and 85-kDa bands and ACT in the 70-kDa band. The ACT- and DHOD-specific signal intensities may be roughly normalized using the 70-kDa band as standard, suggesting that the quantity of the 35-kDa mature DHOD in *B. saliens* may be much larger than the quantity of the DHOD domains in the primary 70-kDa ACT–DHOD translation product. When we applied this method to *B. saliens* ACT, we found that the quantity of the ACT domain in 70-kDa ACT–DHOD was relatively small, suggesting that the level of 35-kDa ACT protein is very low. Alternatively, a small amount of the native 35-kDa ACT raises the possibility that it is susceptible to endogenous proteolysis [17,18]. Probably because of a small quantity of the 85-kDa protein, our extensive searches only detected a DHOD-specific, but not ACT-specific, polypeptide with weak signal intensities (Fig. 4S, C). There are two

possibilities of the components of 85-kDa protein, one is ACT–DHOD (70-kDa) with some moiety (15-kDa), and the other DHOD (35-kDa) with some moiety (50-kDa). In this context, however, we found consensus sequence of the covalent binding with SUMO (small ubiquitin-related modifier) on *B. saliens* ACT domain (Annoura, unpublished). Interestingly, neither *T. cruzi* nor *Leishmania*, which carries independent *ACT* and *DHOD* genes, possesses such a binding site on ACT, suggesting the need for further study of the processing and maturation of ACT, the biological significance of the primary translation product, ACT–DHOD protein, and of independent DHOD and ACT in *B. saliens*.

We attempted to prepare the full-length recombinant ACT–DHOD protein in *Escherichia coli*, resulting in inclusion bodies that were not suitable for the enzymatic assay. However, high levels of 35-kDa mature DHOD exist in *B. saliens* cytosolic fraction (Figs. 1B and 2B) with N-terminal amino acid blocked. An in vitro processing assay clearly indicated that ACT–DHOD was processed by some component(s) in the *B. saliens* lysate in a short time, yielding 35-kDa DHOD. Moreover, the kinetic properties of *B. saliens* recombinant DHOD (V334-E648) is similar to those of the *T. cruzi* DHOD [4]. These results strongly suggested that *B. saliens* 35-kDa DHOD, which resembles *T. cruzi* enzyme, is a functional mature protein and may play an important role for both pyrimidine biosynthesis and fumarate reduction in controlling the cellular redox state.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.04.102.

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